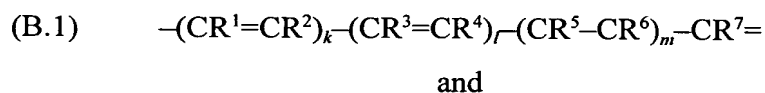


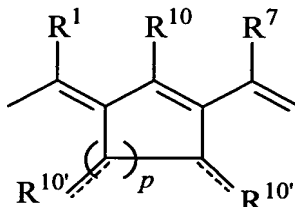
We Claim:

1. A mobility-modifying cyanine dye comprising: (i) a first substituted or unsubstituted parent heteroaromatic benzazole/benzazolium ring system having a linking moiety of the formula $-L-LG$, where L is a linker and LG is a linking group, attached to the heteroaromatic ring nitrogen; (ii) a second substituted or unsubstituted parent heteroaromatic benzazole/benzazolium ring system having a mobility-modifying moiety attached to the heteroaromatic ring nitrogen; and an electron delocalizing bridge connecting the first and second parent benzazole/benzazolium rings *via* their respective C2 carbons, wherein when said mobility-modifying moiety has a net charge of -2 or less or +1 or greater.

2. The mobility-modifying cyanine dye of Claim 1 in which the bridge is a compound selected from the group consisting of:



(B.2)



wherein:

k , l , and m are each independently integers from 0 to 1;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, $-F$, $-Cl$, $-CN$, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl or 5-14 membered heteroaryl;

R^{10} and $R^{10'}$ are each independently selected from the group consisting of hydrogen, oxygen, halogen, $-F$, $-Cl$, $-CN$, $-CF_3$, $-OR$, $-SR$, $-NRR$, (C_1-C_6) alkyl, (C_5-C_{14})

aryl or 5-14 membered heteroaryl, where each R is independently hydrogen or (C₁-C₆) alkyl; and

p is an integer from 0 to 2, where in structural formula (B.2), the dotted lines at substituents R^{10'} represent bonds that may be independently either single bonds or a double bonds, depending upon the identities of the substituents.

3. The mobility-modifying cyanine dye of Claim 2 in which the bridge is a compound of structural formula (B.1).

4. The mobility-modifying cyanine dye of Claim 3 in which the sum of *k*, *l* and *m* is 2.

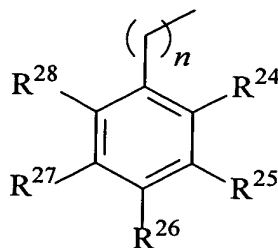
5. The mobility-modifying cyanine dye of Claim 2 in which the bridge is a compound of structural formula (B.1) wherein R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each hydrogen.

6. The mobility-modifying cyanine dye of Claim 1 in which the bridge is $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}=\text{}$.

7. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has a net positive charge.

8. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has a net negative charge.

9. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has the structure:



or a salt thereof, wherein:

5

n is an integer from 1 to 6 (preferably 1 to 3);

R^{24} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{25} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups;

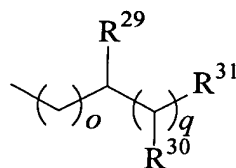
R^{25} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{24} or R^{26} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups;

R^{26} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{25} or R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups; and

R^{27} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{26} or R^{28} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups; and

R^{28} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups.

10. The mobility-modifying cyanine dye of Claim 1 in which the mobility-modifying moiety has the structure:



or a salt thereof, wherein:

5

o is an integer from 1 to 3;

q is an integer from 1 to 3;

R^{29} is a strong anionic substituent, $-S(O)_2O^-$ or $-O-S(O)_2O^-$,

each R^{30} is independently selected from the group consisting of hydrogen a strong anionic substituent, $-S(O)_2O^-$ and $-O-S(O)_2O^-$; and

10

R^{31} is selected from the group consisting of hydrogen, a strong anionic substituent, $-S(O)_2O^-$, $-O-S(O)_2O^-$ and $-CH_3$,

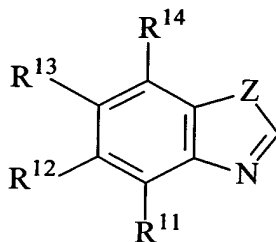
with the proviso that MM has a net charge of at least -2 at a pH in the range of about pH 6 to pH 10.

15

11. The mobility-modifying cyanine dye of Claim 1 in which the first and second heteroaromatic benzazole/benzazolium ring systems are the same.

12. The mobility-modifying cyanine dye of Claim 1 in which the first parent heteroaromatic benzazole/benzazolium ring system has the structure:

20



or a salt thereof, wherein:

25

Z is selected from the group consisting of $-S-$, $-O-$, $-Se-$ and $-CR^8R^9-$, where R^8 and R^9 when taken alone, are each independently (C_1-C_6) alkyl, or when taken together

are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

R¹¹, R¹², R¹³ and R¹⁴, when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W; and

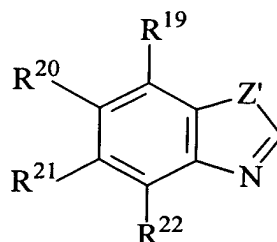
each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

each R is independently -H, -NR''R'', -C(O)R'', -S(O₂)R'', (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R'' is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

13. The mobility-modifying cyanine dye of Claim 1 in which the second parent heteroaromatic benzazole/benzazolium ring system has the structure:



or a salt thereof, wherein:

Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹, where R⁸ and R⁹, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkylene or (C₄-C₅) alkanone;

R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W; and

each W is independently -R-, -X-, =O-, -OR-, =S-, -SR-, -NRR-, =NR-, (C₁-C₆)

perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

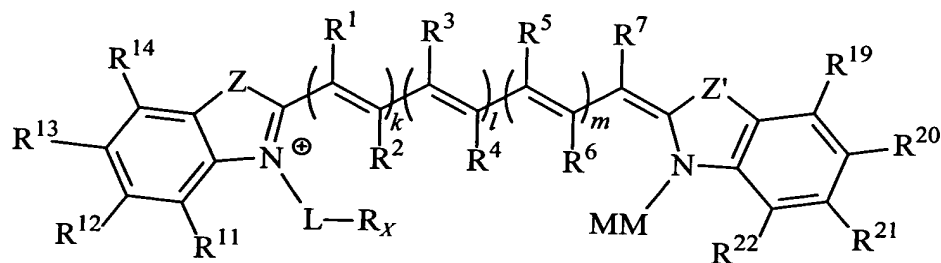
each X is independently a halogen;

each R is independently -H, -NR''R'', -C(O)R'', -S(O)₂R'', (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R'' is independently -H, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

14. A mobility-modifying cyanine dye according to Claim 1 in which the first and second heteroaromatic benzazole/benzazolium rings are each the same or different substituted or unsubstituted indoline/indolinium ring.

15. The mobility-modifying cyanine dye of Claim 1 which has the structure:



or a salt thereof, wherein:

k, *l*, and *m* are each independently integers from 0 to 1;

R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, -CF₃, (C₁-C₆) alkyl, (C₅-C₁₄) aryl and 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

L is a linker;

R_x is a reactive functional group;

Z is selected from the group consisting of -S-, -O-, -Se- and $-CR^8R^9$ -, where R^8 and R^9 when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

5 Z' is selected from the group consisting of -S-, -O-, -Se- and $-CR^8R^9$ -, where R^8 and R^9 , when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkano;

10 R^{11} , R^{12} , R^{13} and R^{14} , when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

20 or when taken together with an adjacent R^n are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

25 R^{19} , R^{20} , R^{21} and R^{22} , when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl

independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

each X is independently a halogen;

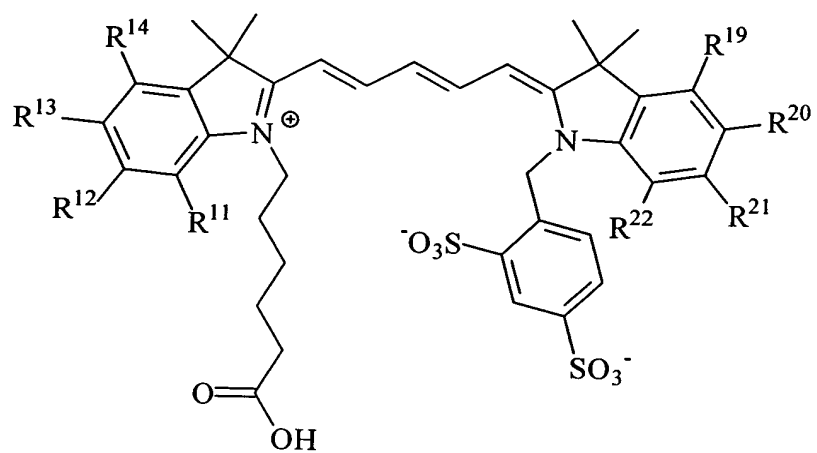
each R is independently -H, -NRⁿRⁿ, -C(O)Rⁿ, -S(O)₂Rⁿ, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each Rⁿ is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl.

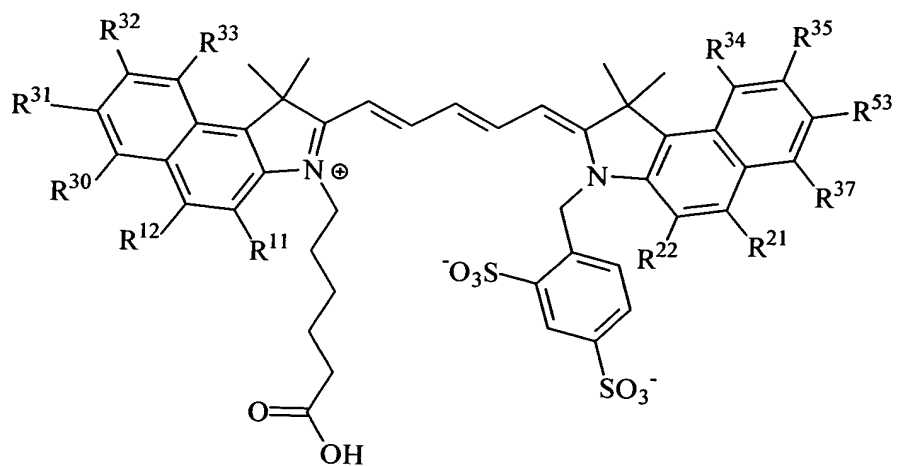
16. The mobility-modifying cyanine dye of Claim 15 in which Z is -NR⁸R⁹-, where R⁸ and R⁹ are each independently (C₁-C₆) alkano; and Z' is -NR⁸R⁹-, where R⁸ and R⁹ are each independently (C₁-C₆) alkano.

17. The mobility-modifying cyanine dye of Claim 15 which is selected from the group consisting of:

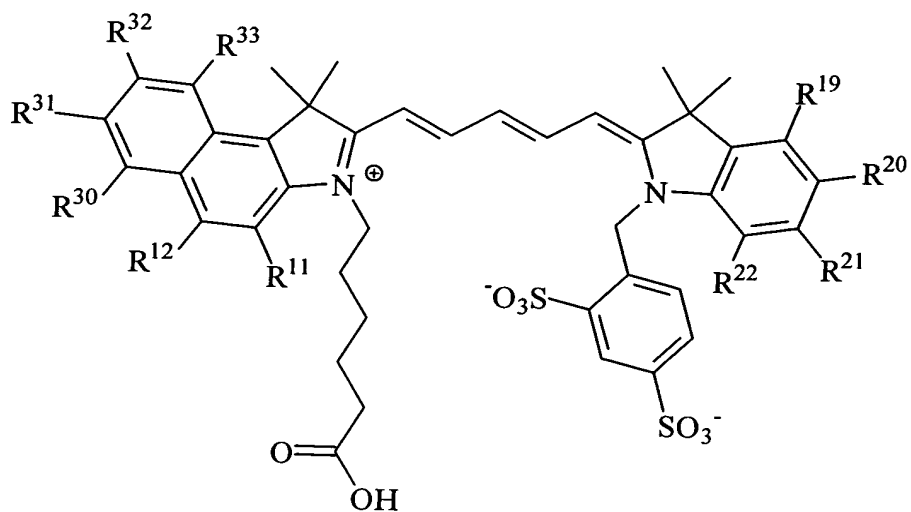
(I.D)



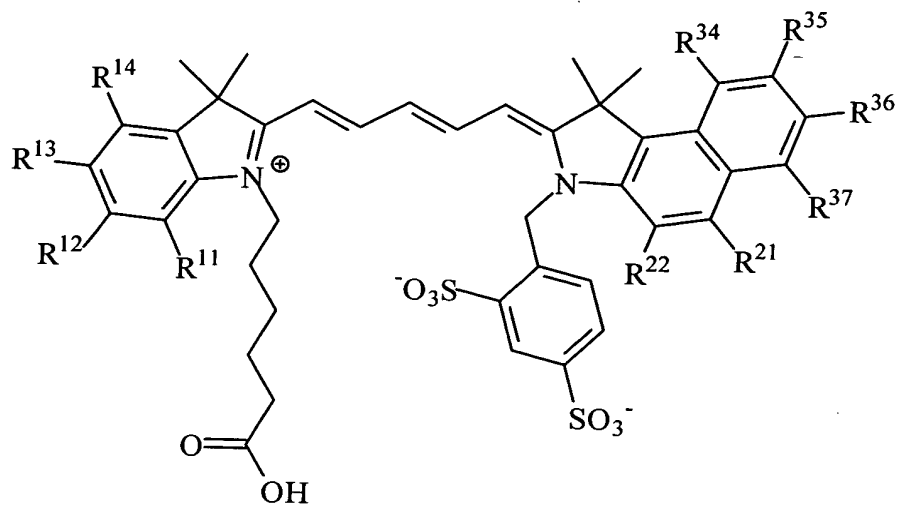
(I.E)



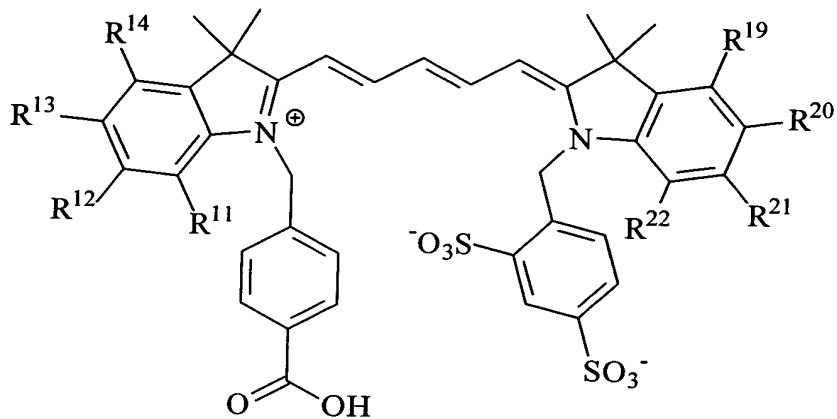
(I.F)



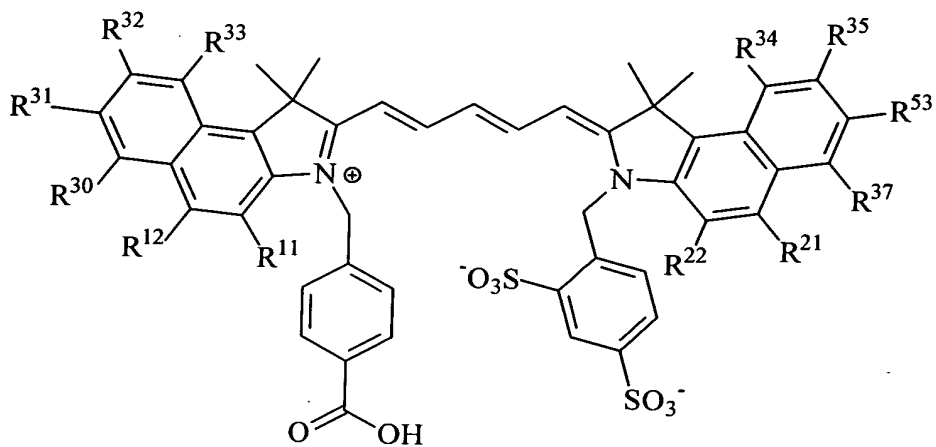
(I.G)

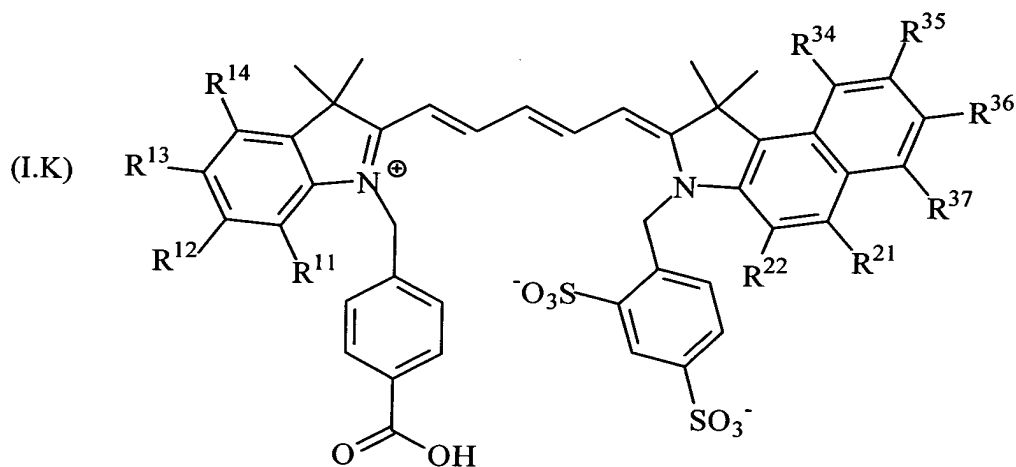
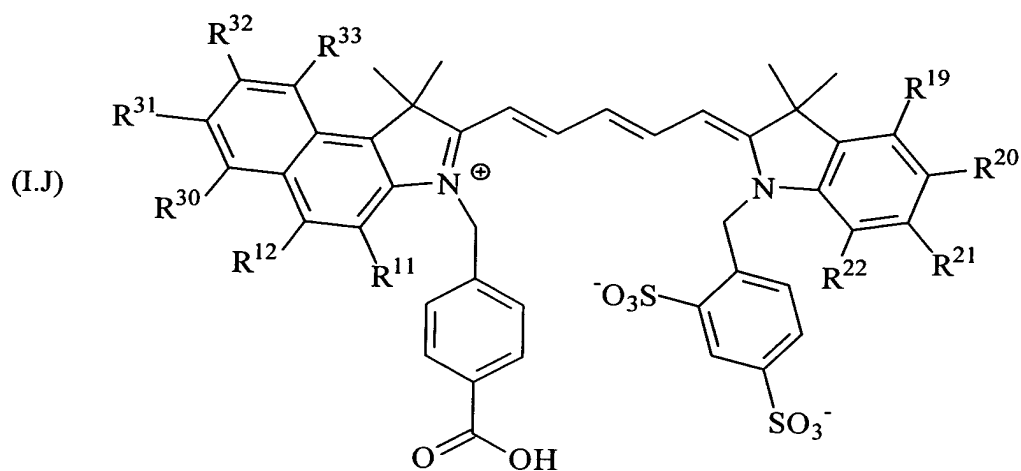


(I.H)



(I.I)

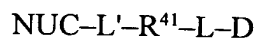




or a salt thereof, wherein:

R^{11} , R^{12} , R^{13} , R^{14} , R^{19} , R^{20} , R^{21} , R^{22} , R^{30} , R^{31} , R^{32} , R^{33} , R^{34} , R^{35} , R^{36} and R^{37} are each independently selected from the group consisting of hydrogen, $-S(O)_2O^-$ and $-O-S(O)_2O^-$.

18. A labeled nucleoside/tide or nucleoside/tide analog having the structure:



or a salt thereof, wherein:

D is a mobility-modifying cyanine dye chromophore;

L is a first linker which is attached to D at a heteroaromatic ring nitrogen;
R⁴¹ is a covalent linkage;
NUC is a nucleoside/tide or nucleoside/tide analog; and
L' is a second linker which is attached to the nucleobase or sugar moiety of

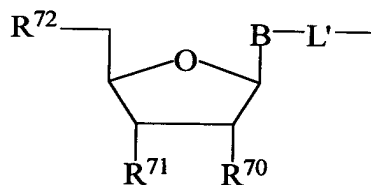
5 NUC.

19. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is enzymatically incorporable.

10 20. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is a terminator.

21. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 which is enzymatically extendable.

22. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 in which -L'-NUC taken together has the structure:



or a salt thereof, wherein:

B is a nucleobase;

25 L' is (C₁-C₂₀) alkylidyl, (C₁-C₂₀) alkyleno, (C₂-C₂₀) alkyno, (C₂-C₂₀) alkeno 2-20 membered heteroalkylidyl, 2-20 membered heteroalkyleno, 2-20 membered heteroalkyno or 2-20 membered heteroalkeno;

R⁷⁰ and R⁷¹, when taken alone, are each independently selected from the group consisting of hydrogen, hydroxyl and a moiety which blocks polymerase-mediated template-directed polymerization, or when taken together form a bond such that the

illustrated sugar is 2',3'-didehydroribose; and

R^{72} is selected from the group consisting of hydroxyl, a phosphate ester

having the formula $\text{---O---}\left[\text{P}\left(\text{O}\right)\left(\text{O}^-\right)\right]_a\text{---O---P}\left(\text{O}\right)\left(\text{O}^-\right)\text{---OH}$, where a is an integer from 0 to 2 and a

phosphate ester analog.

5

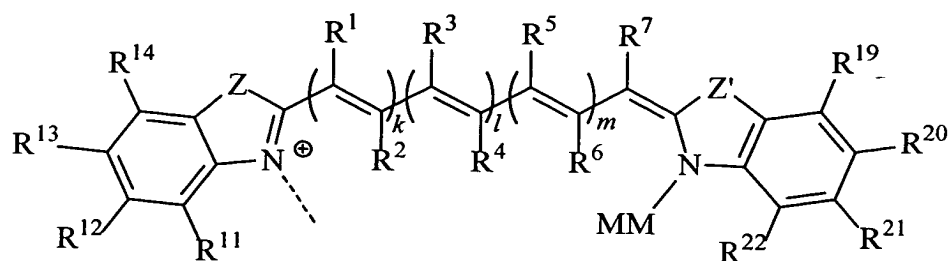
23. The labeled nucleoside/tide or nucleoside/tide analog of Claim 22 in which L' is selected from the group consisting of:

$\text{---C}\equiv\text{C---CH}_2\text{---}$, where the terminal sp carbon is covalently attached to nucleobase B and the terminal methylene (sp^3) carbon is covalently attached to R^{41} ; and

$\text{---C}\equiv\text{C---CH}_2\text{---O---CH}_2\text{---CH}_2\text{---NR}^{47}\text{---R}^{48}\text{---}$, where R^{47} is hydrogen or $(C_1\text{---}C_6)$ alkyl and R^{48} is $\text{---C(O)---(CH}_2)_r\text{---}$, $\text{---C(O)---CHR}^{49}\text{---}$, $\text{---C(O)---C}\equiv\text{C---CH}_2\text{---}$ or $\text{---C(O)---}\phi\text{---(CH}_2)_r\text{---}$, where each r is independently an integer from 1 to 5 and ϕ is C_6 arylidyl or 6-membered heteroarylidyl and R^{49} is hydrogen, $(C_1\text{---}C_6)$ alkyl or a side chain of an encoding or non-encoding amino acid, and where the terminal sp carbon is covalently attached to nucleobase B and the other terminal group is covalently attached to R^{41} .

24. The labeled nucleoside/tide or nucleoside/tide analog of Claim 22 in which nucleobase B is a purine, a 7-deazapurine, a pyrimidine, a normal nucleobase or a common analog of a normal nucleobase.

25. The labeled nucleoside/tide or nucleoside/tide analog of Claim 18 in which D has the structure:



or a salt thereof, wherein:

5

k , l , and m are each independently integers from 0 to 1;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are each independently selected from the group consisting of hydrogen, halogen, $-F$, $-Cl$, $-CN$, $-CF_3$, (C_1-C_6) alkyl, (C_5-C_{14}) aryl or 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

Z is selected from the group consisting of $-S-$, $-O-$, $-Se-$ and $-CR^8R^9-$, where R^8 and R^9 when taken alone, are each independently (C_1-C_6) alkyl, or when taken together are (C_4-C_5) alkylidene or (C_4-C_5) alkanone;

Z' is selected from the group consisting of $-S-$, $-O-$, $-Se-$ and $-CR^8R^9-$, where R^8 and R^9 , when taken alone, are each independently (C_1-C_6) alkyl, or when taken together are (C_4-C_5) alkylidene or (C_4-C_5) alkanone;

R^{11} , R^{12} , R^{13} and R^{14} , when taken alone, are each independently selected from the group consisting of hydrogen, (C_1-C_6) alkyl, (C_1-C_6) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C_5-C_{10}) aryl, (C_5-C_{10}) aryl independently substituted with one or more W, (C_5-C_6) arylalkyl, (C_5-C_6) arylalkyl independently substituted with one or more W, (C_6-C_{16}) arylalkyl, (C_6-C_{16}) arylalkyl independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

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or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

5 R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

10 or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

20 each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

25 each X is independently a halogen;

each R is independently -H, -NRⁿRⁿ, -C(O)Rⁿ, -S(O)₂Rⁿ, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl;

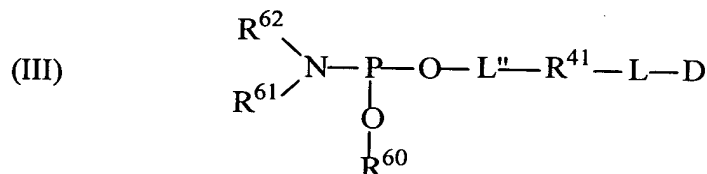
30 each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each Rⁿ is independently -H, (C₁-C₆) alkyl, (C₁-C₆)

alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arlyalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

the dotted line at the heteroaromatic ring nitrogen indicates the point of attachment of linker L.

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26. A mobility-modifying phosphoramidite reagent having the structure:



10

or a salt thereof, wherein:

N, O and P are nitrogen, oxygen and phosphorous, respectively;

D is a mobility-modifying dye chromophore or a protected derivative thereof;

L is a first linker;

R⁴¹ is a bond or a covalent linkage;

L'' is a bond or a second linker;

R⁶⁰ is a phosphite ester protecting group;

R⁶¹, when taken alone, is selected from the group consisting of (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶² forms a straight-chain or branched (C₂-C₁₀) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno; and

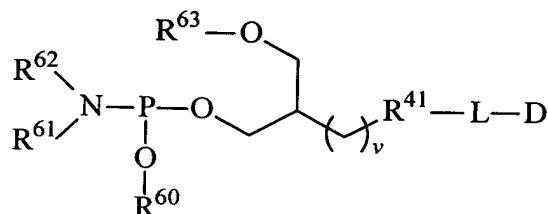
R⁶², when taken alone, is selected from the group consisting of (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶¹ forms a straight-chain or branched (C₂-C₁₀) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno.

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27. The mobility-modifying phosphoramidite reagent according to Claim 26 which has the structure:

(III.A)



wherein:

N, P and O are nitrogen, phosphorous and oxygen, respectively;

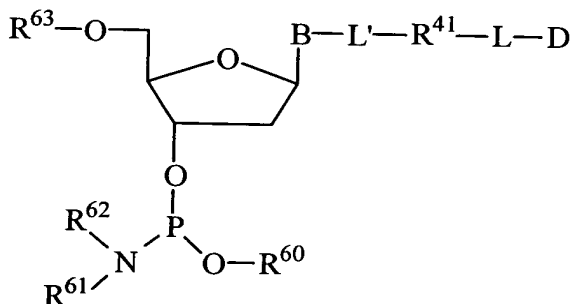
R⁴¹, L, D, R⁶⁰, R⁶¹ and R⁶² are as previously defined;

R⁶³ is hydrogen or an acid-labile hydroxyl protecting group; and

v is an integer from 1 to 30.

28. A mobility-modifying phosphoramidite reagent having the structure:

(IV)



or a salt thereof, wherein:

O, P and N are oxygen, phosphorous and nitrogen, respectively;

B is a nucleobase or a protected derivative thereof;

D is a mobility-modifying dye chromophore or a protected derivative thereof;

L is a first linker which is attached to D at a heteroaromatic ring nitrogen;

R⁴¹ is a bond or a covalent linkage;

L' is a bond or a second linker;

R⁶⁰ is a phosphite ester protecting group;

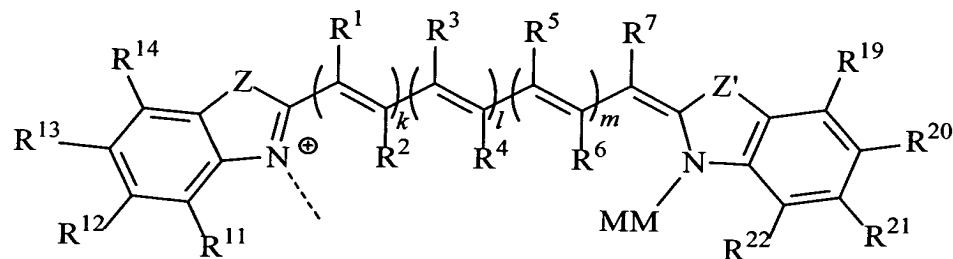
R⁶¹, when taken alone, is selected from the group consisting of (C₁-C₆)

alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶² forms a straight-chain or branched (C₂-C₁₀) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno; and

R⁶², when taken alone, is selected from the group consisting of (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₃-C₁₀) cycloalkyl, (C₅-C₂₀) aryl and (C₆-C₂₆) arylalkyl, or when taken together with R⁶¹ forms a straight-chain or branched (C₂-C₁₀) alkyleno or a straight-chain or branched 2-10 membered heteroalkyleno; and

R⁶³ is hydrogen or an acid-labile hydroxyl protecting group

29. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which D has the structure:



or a salt thereof, wherein:

k, *l*, and *m* are each independently integers from 0 to 1;

R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each independently selected from the group consisting of hydrogen, halogen, -F, -Cl, -CN, -CF₃, (C₁-C₆) alkyl, (C₅-C₁₄) aryl or 5-14 membered heteroaryl;

MM is a mobility-modifying moiety;

Z is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹ when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkanos;

Z' is selected from the group consisting of -S-, -O-, -Se- and -CR⁸R⁹-, where R⁸ and R⁹, when taken alone, are each independently (C₁-C₆) alkyl, or when taken together are (C₄-C₅) alkyleno or (C₄-C₅) alkanos;

R¹¹, R¹², R¹³ and R¹⁴, when taken alone, are each independently selected

from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

R¹⁹, R²⁰, R²¹ and R²², when taken alone, are each independently selected from the group consisting of hydrogen, (C₁-C₆) alkyl, (C₁-C₆) alkyl independently substituted with one or more W, 2-6 membered heteroalkyl, 2-6 membered heteroalkyl independently substituted with one or more W, (C₅-C₁₀) aryl, (C₅-C₁₀) aryl independently substituted with one or more W, (C₅-C₆) arylaryl, (C₅-C₆) arylaryl independently substituted with one or more W, (C₆-C₁₆) arylalkyl, (C₆-C₁₆) independently substituted with one or more W, 6-16 membered arylheteroalkyl, 6-16 membered arylheteroalkyl independently substituted with one or more W, 5-10 membered heteroaryl, 5-10 membered heteroaryl independently substituted with one or more W, 5-6 membered heteroaryl-heteroaryl, 5-6 membered heteroaryl independently substituted with one or more W, 6-16 membered heteroarylalkyl, 6-16 membered heteroarylalkyl independently substituted with one or more W, 6-16 membered heteroaryl-heteroalkyl and 6-16 membered heteroaryl-heteroalkyl independently substituted with one or more W,

or when taken together with an adjacent Rⁿ are each independently selected from the group consisting of (C₆-C₁₀) aryleno, (C₆-C₁₀) aryleno independently substituted

with one or more W, 6-10 membered heteroaryleno and 6-10 membered heteroaryleno independently substituted with one or more W;

each W is independently -R, -X, =O, -OR, =S, -SR, -NRR, =NR, (C₁-C₆) perhaloalkyl, -CX₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -NHOH, -S(O)₂R, -C(O)R, -C(S)R, -C(O)OR', -C(S)OR', -C(O)SR', -C(S)SR', and -C(NR)NRR, wherein:

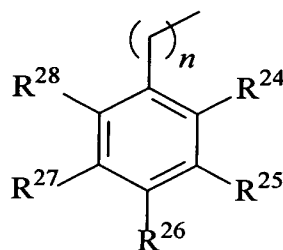
each X is independently a halogen;

each R is independently -H, -NR''R'', -C(O)R'', -S(O₂)R'', (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl;

each R' is independently (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkenyl and (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and each R'' is independently -H, (C₁-C₆) alkyl, (C₁-C₆) alkanyl, (C₂-C₆) alkynyl, (C₅-C₁₀) aryl, (C₆-C₁₆) arylalkyl, 5-10 membered heteroaryl or 6-16 membered heteroarylalkyl; and

the dotted line at the heteroaromatic ring nitrogen indicates the point of attachment of linker L.

30. The mobility-modifying phosphoramidite reagent of Claim 29 in which MM has the structure:



or a salt thereof, wherein:

n is an integer from 1 to 6 (preferably 1 to 3);

R²⁴, when taken alone, is hydrogen, a strong anionic substituent, -S(O)₂O⁻,

or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{25} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups;

5 R^{25} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{24} or R^{26} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups;

10 R^{26} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{25} or R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups; and

15 R^{27} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{26} or R^{28} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups; and

20 R^{28} , when taken alone, is hydrogen, a strong anionic substituent, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$, or when taken together with R^{27} is a benzo group or a benzo group independently substituted with one or more strong anionic substituents, $-\text{S}(\text{O})_2\text{O}^-$, or $-\text{O}-\text{S}(\text{O})_2\text{O}^-$ groups.

31. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which L is $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$ (pentano).

25 32. The mobility-modifying phosphoramidite reagent according to Claim 26, 27 or 28 in which R^{41} is a covalent linkage formed upon the reaction between an electrophile and a nucleophile.

30 33. The mobility-modifying phosphoramidite reagent according to Claim 31 in which R^{41} has the structure $-\text{C}(\text{O})-\text{NR}^{56}-$, where R^{56} is hydrogen or (C_1-C_6) alkyl.

34. A polynucleotide labeled with a mobility-modifying dye according to

Claim 1.

5 35. A method of generating a labeled primer extension product, comprising the step of enzymatically extending a primer-target hybrid in the presence of a mixture of enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a mobility-modifying dye according to Claim 1.

10 36. The method of Claim 35 in which the terminator is a mixture of four different terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U and wherein at least one of the terminators is labeled with a mobility-modifying dye according to Claim 1.

15 37. The method of Claim 36 in which each of the four different terminators is labeled with a different, spectrally-resolvable fluorophore, and one of the terminators is selected from the group consisting of Compound **29** and Compound **32**.

20 38. A kit for generating a labeled primer extension product, comprising enzymatically-extendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a mobility-modifying cyanine dye according to Claim 1.

25 39. The kit of Claim 38 in which the terminator is a set of four different mobility-modified terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U.

30 40. The kit of ~~Claim 39~~ in which the set of four different terminators is a set of mobility-matched terminators.

41. The kit of Claim 39 in which the set of mobility-matched terminators comprises Compounds **31**, **32**, **33** and **34**.